AD_____

Award Number: W81XWH-11-1-0527

TITLE: Collaborative Model for Acceleration of Individualized Therapy of Colon Cancer

PRINCIPAL INVESTIGATOR: Aik Choon Tan, Ph.D.

CONTRACTING ORGANIZATION:
UNIVERSITY OF COLORADO, DENVER
AURORA CO 80045-2505

REPORT DATE: OCTOBER 2014

TYPE OF REPORT: ANNUAL

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
OCTOBER 2014	ANNUAL Report	15 SEP 2013 – 14 Sep 2014

4. TITLE AND SUBTITLE

Collaborative Model for Acceleration of Individualized Therapy of Colon Cancer

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-11-1-0527

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Aik Choon Tan, Ph.D., S. Gail Eckhardt, M.D. and Todd M. Pitts, M.S.

E-Mail: aikchoon.tan@ucdenver.edu;gail.eckhardt@ucdenver.edu;todd.pitts@ucdenver.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

UNIVERSITY OF COLORADO, DENVER

AURORA CP 80045-2505

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Colorectal cancer (CRC) represents a major health burden, and is the third leading cause of cancer deaths in the U.S. In the past decade, the median survival among patients with metastatic CRC has significantly improved, primarily due the development of active chemotherapeutic regimens that include biological agents. However, despite this success, patients soon run out of therapeutic options and receive salvage therapy that results in only a few weeks of disease stability. We have proposed to employ a team science, systems biology based approach to rapidly identify novel anti-cancer agents and individualize therapeutic strategies in preclinical CRC models. In this Year 3 Progress report, we will present the tasks and key accomplishments achieved within this period of time. In brief, we have completed all the tasks in Aim 1, and task 2 in Aim 2 in Year 3. We will continue to work on task 1 in Aim 2 for the NCE in Year 4.

15. SUBJECT TERMS
Colorectal cancer, novel anti-cancer agents, bioinformatics, next-generation sequencing, predictive biomarkers, individualized medicine

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	8	19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

Introduction: Colorectal cancer (CRC) represents a major health burden, and is the third leading cause of cancer deaths in the U.S. In the past decade, the median survival among patients with metastatic CRC has significantly improved, primarily due the development of active chemotherapeutic regimens that include biological agents. However, despite this success, patients soon run out of therapeutic options and receive salvage therapy that results in only a few weeks of disease stability. This is particularly true for a subset of patients that have a mutation in the KRAS gene, since it has been shown that one of these new treatments is not effective for them. Therefore, new agents are needed that can stabilize disease and hopefully prolong life in patients with CRC. One of the lessons learned in CRC, in fact, in patients with the KRAS mutation in their tumor, is the importance of not only developing new effective drugs, but also developing ways to select patients for those treatments. Unfortunately the lack of such strategies is what led to thousands of CRC patients with KRAS mutations being treated with epidermal growth factor receptor (EGFR) inhibitors at considerable toxicity and no benefit, when it was discovered that tumors with this mutation did not respond to these drugs. This new area of patient selection, or individualized therapy, is based upon a robust set of research tools in the field of bioinformatics. Therefore, successful research teams are comprised of clinicians, who treat patients with cancer, and bioinformaticians, that are able to synthesize large sets of data and look for patterns of response or resistance to a particular new drug. Such a team has been assembled for this proposal. Thus, the overall goal of this Idea Award is enhance the efficiency and speed of developing novel and individualized therapy for patients with KRAS mutant colorectal cancer (CRC) using a comprehensive bioinformatics approach and novel preclinical models of human CRC. This proposal has the potential of providing novel, individualized therapeutic strategies for CRC patients with KRAS mutations that are poised for clinical testing at the completion of this work. The yield will be highly relevant, as new drug development will not only be jump-started by this proposal but agents to be tested clinically will be tailored for specific populations of patients with CRC, thereby potentially conferring greater clinical benefit. In this progress report, we will describe our research achievements and outcomes for **Year 3**.

Aim 1. To develop predictive classifiers for 3 novel agents using preclinical models of colorectal cancer (CRC).
We have identified the following three novel agents to develop predictive classifier using preclinical models of CRC and these agents will be tested in **Aim 2**.

Table 1: Three novel anti-cancer agents selected in this study.

Agents	Targets	Company	Clinical Developmental Phase
MLN8237 (alisertib)	Aurora Kinase A (AURKA)	Millennium Pharmaceuticals/Takeda	Phase I/II
MLN0128	TORC1/TORC2	Millennium Pharmaceuticals/Takeda	Phase I
ENMD2076	Aurora Kinase A (AURKA) and Angiogenic Kinase (KDR)	EntreMed	Phase I/II

In Year 3, we have completed all the tasks described in Aim 1. The tasks are previously reported in our Year 2 progress report. Here we would like to highlight the tasks in **Aim 2** we have completed in Year 3.

Aim 2. To validate the preclinical efficacy of these classifiers against 20 independent patient-derived CRC explant models.

Task 1: Prediction of the human CRC explants (Months 24-36, Drs. Eckhardt and Tan)

We have initiated the classifiers development for these anti-cancer agents. As we learned more about the cancer biology of CRC, it has became clear not all CRC carried the same molecular mutations would response to the same compounds. For example, KRAS mutation was found in ~45% of CRC, however, recent studies have demonstrated that not all KRAS mutant CRC models are addicted to KRAS. KRAS mutant could be sub-classified based on their KRAS-dependency into KRAS-dependent and KRAS-independent. In Year 3, we have worked in this research to better define KRAS-dependency in CRC, using MEK inhibitor as a pharmacological surrogate. Here, we highlight some findings from our work in this area. We used a novel MEK inhibitor (TAK-733, one of our initial anti-cancer compound). As MEK1/2 is a downstream signaling of the RAS/RAF pathway, we hypothesize that if we could develop a MEK inhibitor signature, this signature could be used to stratify patients based on KRAS-dependency and predicting sensitivity to MEK inhibitor. To develop the classifier specific for KRAS-mut CRC, we collected various MEK inhibitors that we have treated in our panel of CRC KRAS mut cell lines. As illustrated in Table 2, we used four MEK inhibitors (TAK-733, AZD6244, PD0325901 and U0126). We define KRAS mut cell lines as sensitive if the cell line is sensitive in at least two MEK inhibitors. Similarly, we define KRAS mut cell lines as resistant if the cell line is resistant to at least two MEK inhibitors.

Table 2: CRC KRAS mut cell lines sensitive and resistant across four MEK inhibitors. S and R represent sensitive and resistant to the MEK inhibitor. nd denotes not determine in the particular study.

CRC Cell Lines	TAK733 (This Paper)	AZD6244 (Tentler et al MCT2010)	PD-901 (Pitts et al PLoS ONE2014)	U0126 (Flanigan et al CCR2013)
LOVO	S	S	S	nd
SKCO1	S	S	nd	nd
LS513	S	S	nd	S
SW403	S	S	nd	nd
LS1034	S	S	S	nd
SW620	S	S	nd	S
LS123	R	R	R	R
HCT15	nd	R	R	R
DLD1	R	nd	R	nd
GP2D	R	nd	R	nd
T84	R	nd	nd	nd

We used Significance Analysis of Microarrays (SAM) to define MEK sensitivity signature in sensitive (LOVO, SKCO1, LS513, SW403, LS1034 and SW620) and resistant (LS123, HCT15, DLD1, GP2D and T84) KRAS mut cell lines. We found 118 genes differentially expressed between MEK sensitive and resistant lines (Figure 1A). Using the signature, we cluster 11 KRAS mut CRC explants to predict their sensitivity against MEK inhibitor. As illustrated in Figure 1B, we found that 7 explants are clustered together with the MEK sensitive and 4 models were clustered with the MEK resistant models.

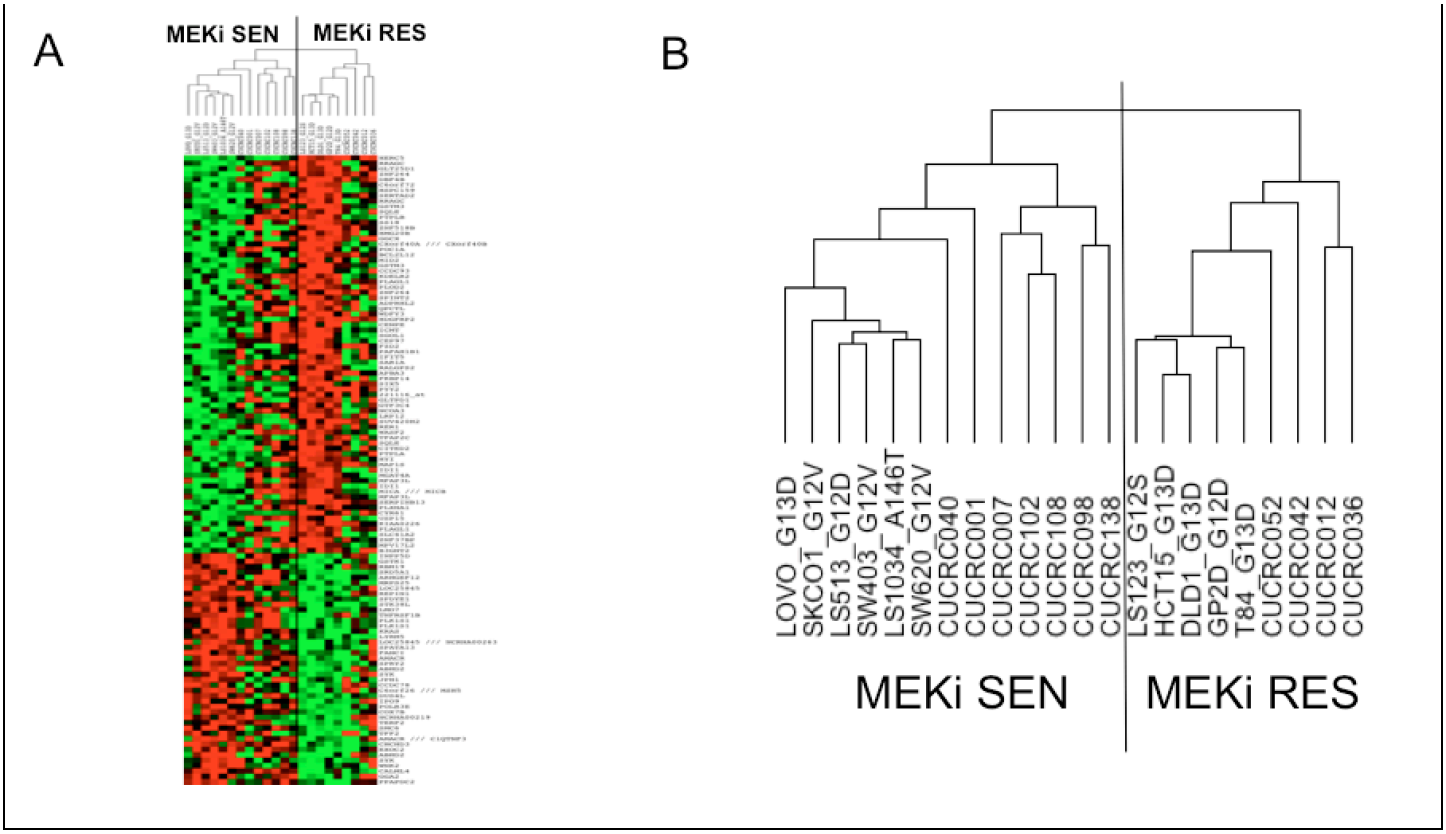

Figure 1. MEK sensitivity signature. A) 118 genes identified as MEK sensitive and resistant cell lines. B) Zoom-in the clustering of the 11 CRC KRAS mut explants.

Table 3: TAK-733 responses of the 11 KRAS mut CRC explants.

Explants	TAK-733 Response (TGII < 20% = S; TGII > 20% = R)	MEKi Signature (This Paper)
CUCRC012	S	S
CUCRC001	S	S
CUCRC102	S	R
CUCRC040	S	S
CUCRC052	S	R
CUCRC098	S	S
CUCRC108	S	S
CUCRC138	S	S
CUCRC007	R	S
CUCRC042	R	R
CUCRC036	R	R

When we tested the TAK-733 sensitivity against the 11 KRAS mut CRC explants (Table 3), we found that the signature prediction is 73% (8 out 11 correct prediction). The accuracy of predicting MEK sensitive is 86% whilst the accuracy of predicting MEK resistant is only 50%. This indicates that the signature is more accurate in predicting MEK sensitive models. We are working on improving the signature and applying the same concept to develop the other predictors for the anti-cancer compounds in this project. This will be completed in the No-cost extension until Sept 2015.

Task 2: The human CRC explants will be treated with the agent and assessed for response (Months 24-36, Dr. Eckhardt).
We have completed the treatment on 20 CRC explants with the selected anti-cancer agents to assess for response, these models will be used to validate the prediction of the classifier in Task 1.

Key Research Accomplishments:

- Completed in vitro screening on a large panel of CRC cell lines to determine the activity of three novel anti-cancer agents
- Completed baseline gene expression profiling of CRC cell lines and patient-derived tumor explants by high-throughput RNA-sequencing approach
- Analyzed the RNA-seq data with bioinformatics pipeline
- Completed treatment of 20 CRC explants.
- Developed initial predictive classifiers the three novel anti-cancer agents

Reported Outcome:

Pitts TM, Newton TP, Bradshaw-Pierce EL, Addison R, Arcaroli JJ, Klauck PJ, Bagby SM, Hyatt SL, Purkey A, Tentler JT, Tan AC, Messersmith WA, Eckhardt SG, Leong S. (2014). Dual Pharmacological Targeting of the MAP Kinase and PI3K/mTOR Pathway in Preclinical Models of Colorectal Cancer. PLoS ONE. (Accepted Oct 29, 2014).

Conclusions: We have completed all the **Tasks in Aim 1,** and currently in the refinement process for the development of the predictive classifiers stage. We also completed the **Task 2 in Aim 2**, and we anticipate in the next 12 months we will have a "lock-down" version of the predictive classifiers for testing in CRC explants. We have identified the three anti-cancer agents to move into **Aim 2** of this project. We anticipate that by the end of Year 4 (NCE), we will complete all the tasks of this proposal.

Pitts TM, Newton TP, Bradshaw-Pierce EL, Addison R, Arcaroli JJ, Klauck PJ, Bagby SM, Hyatt SL, Purkey A, Tentler JT, Tan AC, Messersmith WA, Eckhardt SG, Leong S. (2014). Dual Pharmacological Targeting of the MAP Kinase and PI3K/mTOR Pathway in Preclinical Models of Colorectal Cancer. PLoS ONE. (Accepted Oct 29, 2014).

Flanigan SA, Pitts TM, Newton TP, Kulikowski GN, Tan AC, McManus MC, Spreafico A, Kachaeva MI, Selby HM, Tentler JJ, Eckhardt SG, Leong S. (2013). Overcoming IGF1R/IR Resistance Through Inhibition of MEK Signaling in Colorectal Cancer Models. Clin. Cancer Res. 19(22):6219-6229. [Epub ahead of print, Sep 17, 2013]. [PMID: 24045180]

Tentler JJ, Nallapareddy S, Tan AC, Spreafico A, Pitts TM, Morelli MP, Selby HM, Kachaeva MI, Flanigan SA, Kulikowski GN, Leong S, Arcaroli JJ, Messersmith WA, Eckhardt SG. (2010) Identification of predictive markers of response to the MEK1/2 inhibitor selumetinib (AZD6244) in K-ras-mutated colorectal cancer. Molecular Cancer Therapeutics. 9(12):3351-3362. [Epub 2010 Oct 5.] [PMID: 20923857]